

# FLUORESCENCE MICROSCOPE

## BACKGROUND OF THE INVENTION

### 1. Cross Reference

This application claims priority from Provisional Application Number 60/212,737,  
5 filed on June 20, 2000.

### 2. Field of the Invention

The present invention is directed to a microscope that permits three optical techniques  
in one system. The microscope allows biological samples, as an example, to be viewed in  
three dimension (stereoscopic), two dimension (compound optic) and macro with reflected  
10 light fluorescence and transmitted light brightfield. The microscope permits the user to sort  
samples under stereo fluorescence illumination and to verify structural detail under  
compound optic fluorescence illumination on one instrument. The three position rotating  
nosepiece houses one stereoscopic and two compound lens. All lens are, by way of example,  
parcenter and parfocal.

### 15 3. Related Art

Conventionally, fluorescence equipped stereo microscopes permit users to view  
samples, typically in a magnification range of 10x-120x. If the magnification is sufficient to  
observe the structure in fluorescence, then sorting of the sample is possible. On the other  
hand, if the magnification is insufficient to view the structure in common, each sample must  
20 be taken out of the Petri dish, placed on a microscope slide, and transferred to another high  
magnificaion compound fluorescence microscope for evaluation and selection. The prior art  
thus was extremely tedious and time consuming.

## SUMMARY OF THE INVENTION

Therefore, an object of the present invention is to provide a novel and improved microscope which overcomes all of the problems identified above.

The system according to the invention thus has the following advantages. First, it provides  
5 two-dimension and three-dimension images on one microscope system for both transmitted light brightfield and reflected light fluorescence viewing. A nose piece is provided for one stereo lens and two compound lenses. When the nosepiece rotates to compound lens, the microscope automatically shifts left to single optical axial system — while lens remains in center of field of view. Lenses are parcenter and parfocal.

10 The system according to the present invention further provides interchangeable barrier filter sliders on fluorescence filter modules. A magnetic device is provided for proper filter module positioning on the turret.

The system further provides a unique base that offers both tilting mirrors for stereoscopic (3D) microscope use and high aperture lens for compound (2D) imaging with  
15 high power objectives. A shutter system includes Foot pedal controlled shutter for transmitted light, switchable to: a. always open for continuous transmitted light; b. controlled by the foot pedal; or c. OFF — always closed.

According to the present invention, there is provided a microscope having a microscope for viewing samples in stereoscopic and in compound optical images in  
20 transmitted light brightfield and reflected light fluorescence, said microscope comprising: a stereo lens; a compound lens; a nosepiece carrying said stereo lens and said compound lens; stereo microscope body that is shiftable about an axis to be placed properly over the stereo lens or the compound lens; a transmitted light base for providing illumination for transmitted

light brightfield for said stereo and compound lenses; and a prism shift mechanism to create binocular images from a single axis compound image created.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

These and other objects, features, and advantages of the present invention will be  
5 apparent with reference to the following description and drawings.

Figure 1 illustrates a side view of the microscope according to the present invention;

Figure 2 illustrates a top view of the transmitted light base from the microscope of  
Figure 1;

Figure 3 illustrates a rear view of the transmitted light base from the microscope of  
10 Figure 1;

Figure 4 illustrates a top view of the auto prism shift assembly from the microscope of  
Figure 1;

Figure 5 illustrates a bottom view of the nosepiece (objective turret) from the  
microscope of Figure 1;

15 Figure 6 illustrates a top view of a quadruple filter turret assembly and filter module  
from the microscope of Figure 1; and

Figure 7(a), (b), (c) and (d) illustrate further views of the filter module from the  
microscope of Figure 1.

### **DETAILED DESCRIPTION OF THE INVENTION**

20 Referring to the figures, the microscope according to a preferred embodiment of the  
present invention, permits the users to view and sort biological samples, for example,  
microscopic animals including worms, fish embryos, fruit fly embryos, etc., that exhibit

fluorescence in as small an area as a single cell, and to be able to do this procedure rapidly.  
The samples typically are held in a Petri dish with hundreds of animals present.

Figure 1 is a side view of the microscope. The microscope includes a transmitted light  
base 1, compound objective (objective turret) 2, focus drive 3, an auto prism shift mechanism  
5 4, viewing head 5, eyepieces 6, GFP Quad turret illuminator 7, stereo microscope body 8,  
objective turret with automatic shift 9, and a stereo objective 10.

Figure 2 shows a top view of the transmitted light base 1. It includes a control knob  
for selecting condensor or mirror (with aperture control) 11, aperture diaphragm 12, power  
supply 13, fiberoptic cable 14, solenoid switch 15, switch box 16, high power condensor 17,  
10 frosted mirror 18, adjustment knob for mirror tilt 19, and a plain mirror 20.

Figure 3 shows a rear view of the transmitted light base 1, which further includes a  
fiberoptic bundle 21, switch box 22, solenoid 23, and a power supply 24.

Figure 4 is a top view of an auto prism shift assembly 4. The assembly includes a  
special combining prism 25, linkage for prism adjustment 26, magnet for quick release 27,  
15 and flexible cable for auto shift mechanism attaches to nosepiece 28.

Figure 5 is a bottom view of the nosepiece (objective turret) 9. It includes a stereo  
objective receptacle 29, auto axial shift mechanism 30, and two receptacles for two  
compound objectives 31.

Figure 6 depicts a top view of a quadruple filter turret assembly and filter module,  
20 including permanent magnets 32, 34, filter module 33, and filter turret 35.

Figures 7a, 7b, 7c and 7d illustrate various filter modules, including filter module 36,  
a safety key 37 and a barrier filter slider 38.

In accordance with the present invention, the transmitted light base 1 allows for both stereoscopic and compound brightfield illumination. The two tilting mirrors 18, 20 (Figure 2), one shiny and one diffused, allow for varied stereo illumination. The high aperture transmitted light condenser 17 (Figure 2) for the compound optic is located on the slider.

5 This has an aperture diaphragm control 11.

The microscopic system combines the capabilities of a stereo fluorescence microscope and the optics of a compound microscope for fluorescence on one system. Both two-dimensional and three-dimensional images are on one system. It permits fluorescence observations in the stereoscopic mode (mag. 10x-120x). In addition the system has a  
10 nosepiece (objective turret) 9 which can hold two infinity corrected high magnification, long working distance lenses 31 (mag. possible up to 700x) as well as the stereo lens 29 (Figure 5). When either of these lenses is rotated into the optical path, the stereo microscope optical system 8 (Figure 1) shifts to the left while the objective remains over the center of the optical field. This allows the optical center to remain constant and the compound objective now  
15 functions through the right side optical axis of the stereo microscope. This new resulting 2-D high magnification fluorescence image is then split with a prism 25 (Figure 4) to create binocular observation.

The fluorescence filter modules (double cubes) 36 are housed in the circular chamber with a 4-position turret 7 (Figures 1 and 6). The filter modules have an excitation filter; one  
20 dichroic mirror and two interchangeable barrier filters on the slider 38 (Figure 7A). The use of the single, 100% reflecting dichroic mirror permits excitation to pass thru the stereo scope lens and/or the two high power lenses as well. The filter modules are held in place on the turret 35 (Figure 6) (in exact placement) with the magnetic device 32, 34.

The invention also has a unique transmitted light base 1. In the stereo microscope mode, there are two tilting mirrors 18, 20 (Figure 2) in base, which reflects the illumination up to the sample. When high magnification transmitted light is required, the base includes the high numerical aperture condenser 17. It slides into the light path and the tilting mirror slides  
5 out. Therefore, on one base, both stereo tilting mirror and high aperture compound condenser are switchable. In addition the system has a foot pedal controlled solenoid shutter 23 to engage transmitted light.

The filter modules can be moved in and out of the path so that the system can function either as a fluorescence scope, or as a clear path optical system with no filters present in the  
10 path. In addition the cubes have a unique design. The barrier filter pair is installed on the slider 38. One filter module has interchangeable barrier filter sets.

For example, one filter module for Green Fluorescence Protein (GFP) excitation:

Filter module has      exciter filter: 470nm

Dichroic mirror:      485nm

15 Two sliders available for emission either 500 LP or 525BP

Sliders are interchangeable so that on one filter module, the user can see either wideband (500 LP) or narrow band (525 BP) GFP by exchanging barrier filter sliders.

With further reference to Figure 1, the system includes the following salient features  
20 to carry out the invention. The system includes the foot pedal controlled solenoid shutter 23 (Figure 3) to engage and disengage the transmitted light base 1. The four-position vertical fluorescence illuminator 7 uses magnets 32, 34 to grab and align filter modules 36 (Figures 7a, 7b, 7c, 7d) into the correct optical position.

The nosepiece (objective turret) 9 is linked to an automated axial shift mechanism 30 (Figure 5). When the stereo lens is in place, it is centered under the dual path of the stereo optics carrier. This permits 3D observation in fluorescence. When the nosepiece is rotated to being either of the compound lenses 31 into position, the optics carrier 8 is automatically shifted so that the single right hand optical pathway of the stereo optics carrier is centered above the compound objective. This now permits a 2D observation in fluorescence. The sample remains parcenter to the field of view since the stereo lens and compound lens each stop in the exact same position. In addition the lenses are adjustable to be parfocal to each other.

The nosepiece 9 is also automatically linked to the prism shift mechanism 4. When the nosepiece 9 is in stereo position, the prism 25 (Figure 4) is automatically out of path. When nosepiece 9 is rotated to compound position, the prism 25 is automatically shifted into position. This prism 25 then takes the single beam path of observable light from the right hand optical path, and splits it into a binocular image for binocular observation. It can be manually slid out of the path to allow 2x light intensity.

Additional detent to position stereo lens 29 center to right hand optical path so that the system can function as a macro single path optical system for image analysis. In addition, the beam split prism 25 can be slid out to offer 2x light intensity for macro observation.

The fluorescence filter modules 36 allows for one filter module to be utilized for all three techniques: stereo 3D, compound 2D observations and macro. The filter module excites only through the right optical path and emits through both, thus providing multi-use capability.

Provisional application (Serial Number 60/212,737, filed June 20, 2000) is incorporated herein by reference for all which it discloses and illustrates.

There has thus been shown and described a microscope which fulfills all of the objects and advantages sought therefore. Many changes, modifications, variations, and other uses and applications of the subject invention will, however, become apparent to those skilled in the art after considering the specification and the accompanying drawings which

5 disclosed preferred embodiments thereof. Also, changes, modifications, variations, and other uses and applications which do not depart from the spirit and scope of the invention are deemed to be covered by the invention which is limited only by the claims which follow.